THE DIFFERENTIAL RADIOSENSITIVITY OF OOGONIA AND OOCYTES AT DIFFERENT DEVELOPMENTAL STAGES OF THE BRINE SHRIMP, *ARTEMIA SALINA*

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The radiosensitivity of germ cells and oocytes varies between different species and is dependent on the age of the animal at the time of irradiation.

Artemia is well suited for genetic or radiobiological studies because it is easily cultured in the laboratory and has a generation time of 25 to 30 days. The development of female germ cells is synchronous as each brood and the stages of their maturation are easily recognizable from external features.

The effects of acute irradiation on adult life span and reproductive performance of *Artemia* were reported by Grosch and Erdman (1955), Metalli and Ballardin (1962, 1972), Cervini and Giavelli (1965) and Squire (1970). Cervini and Giavelli (1965) studied the sensitivity of *Artemia* oocytes at different meiotic stages using hatchability as the criterion of radiation damage. They found that oocytes were more sensitive in prophase I than in metaphase I. However, histological or cytological observations of oogonia and oocytes in ovaries of irradiated *Artemia* have not been reported. The present study was initiated to obtain this information.

MATERIALS AND METHODS

Dry eggs of the California strain (diploid amphigonic) of *Artemia* obtained from the Aquarium Society of San Francisco, Hayward, California, were hatched in artificial sea water and reared to maturity. The culture methods employed were similar to those described previously (Iwasaki, 1970). In the present experiment, three developmental stages of the brine shrimp were used for irradiation; dry eggs, immature animals (6–8 mm in body length) and adults.

For irradiation with ^{60}Co γ-rays, eggs were placed in a small lucite chamber under dry conditions. Young or adult shrimps were put into a cylindrical lucite vessel, 2 cm in diameter, containing artificial sea water about 0.5 cm in depth. Dry eggs were irradiated with 2, 5, 10, 25, 50, 100, 200 and 300 krads of γ-rays at a dose-rate of 10 krads/min. Young and adult shrimps were irradiated with 2, 5, 10 and 20 krads, at a dose-rate of 2 krads/min. After irradiation, eggs were hatched in artificial sea water and were cultured to maturity; irradiated shrimps were kept in culture medium.

To observe histological changes in the gonads after γ-irradiation, shrimps at each dose level were fixed in Bouin's fluid at desired intervals. Serial longitudinal sections, cut in paraffin at a thickness of 5 μ, were stained with Mayer's hemalum and eosin. There were at least 6 animals in each group.
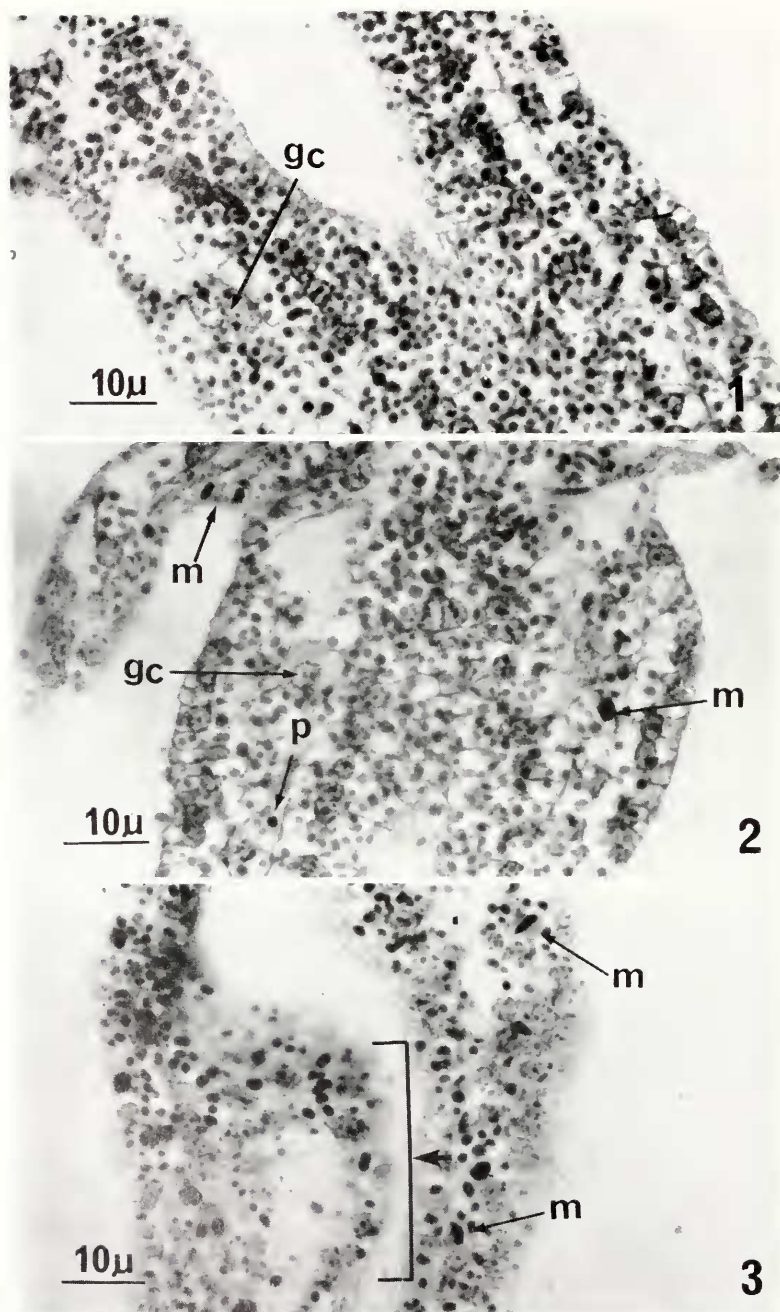
Figure 1. Longitudinal section of a 1-day-old nauplius of *Artemia* hatched from an egg irradiated with 5 krads of γ-rays. The cells appear normal. The germ cell group (gc) consists of 4 to 8 cells, × 1200.

Results

1. *Dry eggs*

Encysted dry eggs temporarily cease development at the gastrula stage and produce thick shells. Their radiosensitivity is extremely low, 50% hatchability requiring a dose of several hundreds krads (Iwasaki, 1964, 1965).

In this experiment, only a dose of 300 krads prevented hatching of dry eggs, but the time required to reach 50% hatching increased considerably as the dose increased from 2 to 200 krads. Nauplii derived from dry eggs irradiated with 50 krads or less reached the mature stage as well as the unirradiated control. γ-ray exposure of 100 krads greatly reduced the viability of the nauplii which hatched from these eggs, and it allowed some individuals to mature but malformations, for example twisted tails, were observed in some animals. In the groups receiving more than 150 krads, essentially all of the animals died within one week.

Microscopic observations indicated that nuclei in the cells of nauplii hatched from eggs irradiated with less than 25 krads were intact and normal in appearance (Fig. 1), but some pycnotic cells were found in nauplii of eggs irradiated with 25 krads or more (Fig. 2). In nauplii hatched from eggs receiving 150 krads, normal mitoses were still found, however, the cells were loosened in appearance (Fig. 3).

No changes were observed in the ovaries of adults hatched from eggs receiving 10 krads or less (Figs. 4a, 4b). When the dose to the eggs was increased to 100 krads, normal growing oocytes were observed in the ovaries of mature survivors, but some pycnotic oocytes were found as well as shown in Figures 5a and 5b.

2. *Immature animals*

The ovary of *Artemia* in this stage, consists of a large population of mitotically active oogonia. Changes in the ovary were apparent at all radiation levels employed, although progressively more damage was present with increasing dose.

In young shrimps receiving a γ-ray dose of 2 krads, some oogonia immediately underwent mitosis, but pycnotic cells also appeared within one day after irradiation as shown in Figure 6. During the first 4 days after irradiation, oogonia degenerated, and the ovary became greatly depleted (Fig. 7). At 10 days to 2 weeks, however, some surviving oogonia began to enter mitosis, and a small number of healthy-appearing oogonia were found (Figs. 8a, 8b); the ovary had increased in size by this time.

The effects of 5, 10 or 20 krads on the cells of the ovary were similar to, but much more pronounced than, those observed at 2 krads. However, following these

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FIGURE 2. Longitudinal section of a 1-day-old nauplius hatched from an egg irradiated with 25 krads. Many mitotic cells (m) and a few cells with pycnotic nuclei (p) are found throughout the body, × 1200.

FIGURE 3. Longitudinal section of a 3-day-old nauplius hatched from an egg irradiated with 200 krads. Arrow indicates an abnormality in the gut lumen. The cells lose their rigidity and many of their structural details. Mitotic cells are still found, × 1200.
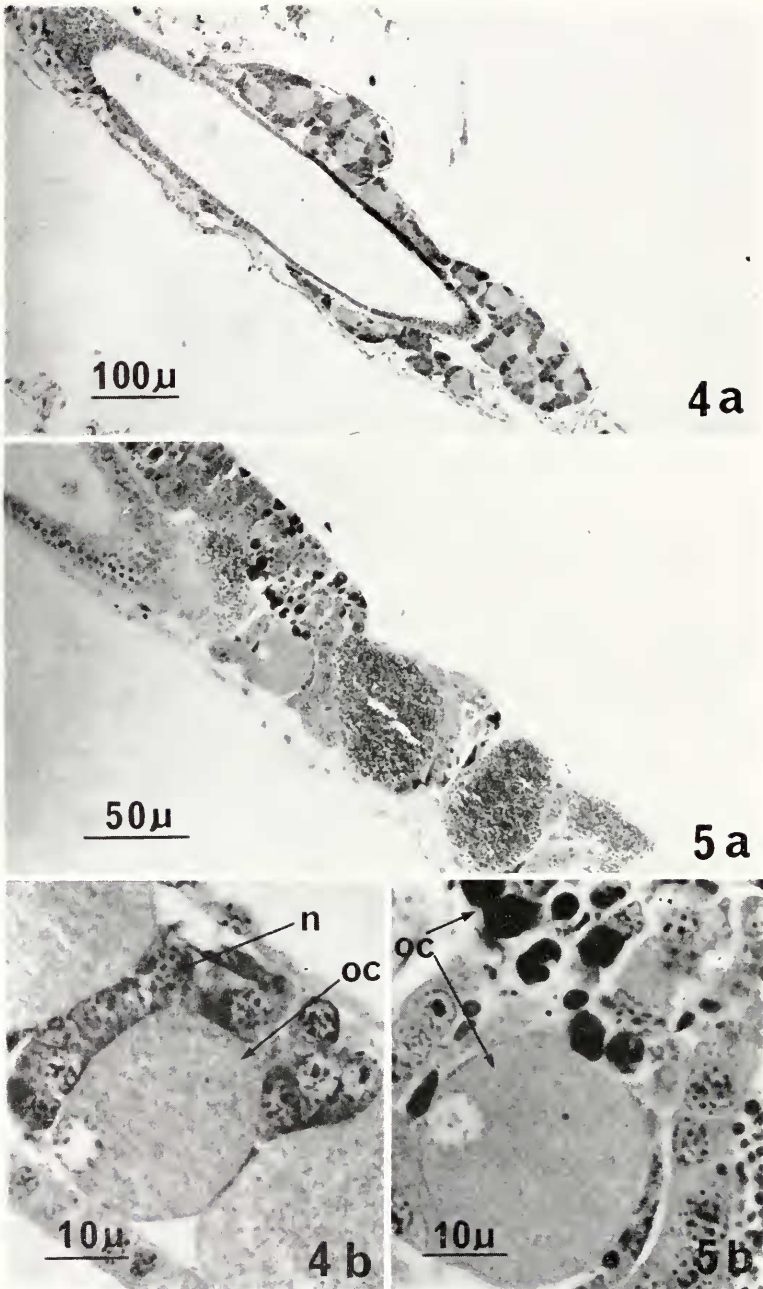
FIGURE 4. Longitudinal section of an ovary of an adult *Artemia* derived from an egg irradiated with 5 krads. Many normal growing oocytes (oc) surrounded by nutritive cells (n) arranged in rows are shown; a: × 120, b: × 1200.

FIGURE 5. Longitudinal section of an ovary of an adult *Artemia* derived from an egg irradiated with 100 krads. The ovary contains degenerated oocytes, nutritive and follicle cells, and normal growing oocytes; a: × 300, b: × 1200.
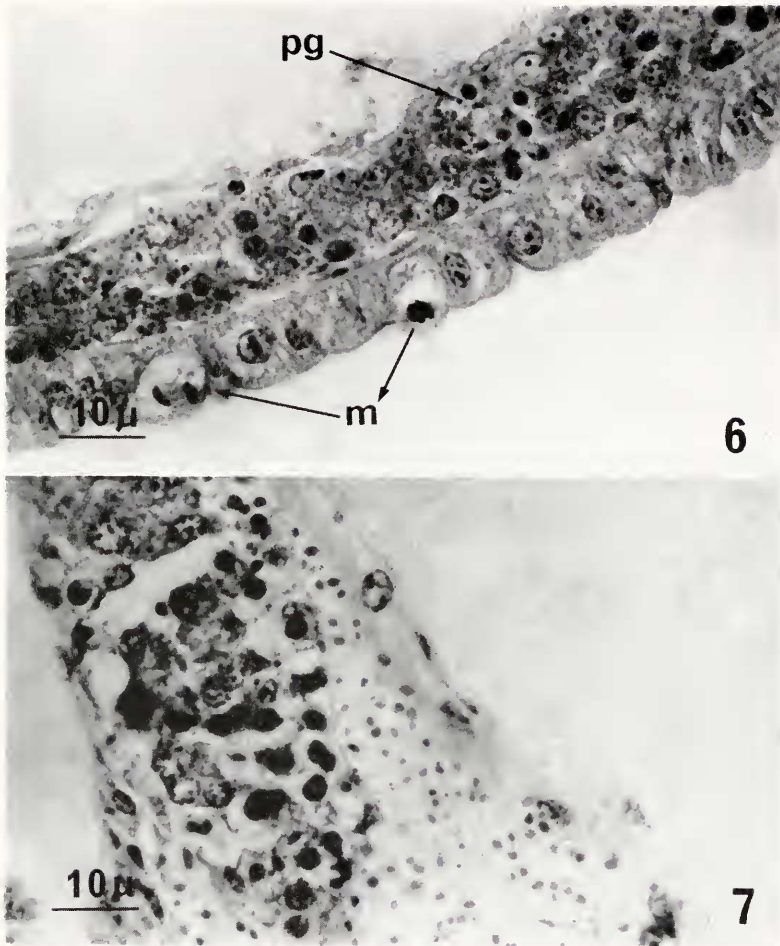
FIGURE 6.   Ovary of a young *Artemia* 1 day after irradiation with 2 krads.   Some pycnotic oogonia (pg) are shown.   Two dividing cells (m) in the gut lumen are also evident, × 1200.

FIGURE 7.   Degenerating ovary of a young *Artemia* 4 days after irradiation with 2 krads. There has been a progressive loss of cells, but a few pycnotic and normal cells are still found, × 1200.

doses, animals were in generally poor condition and usually died before recovery of ovarian damage reached completion.

3. *Adults*

In the normal ovary of an adult shrimp, all prophase oocytes are located along the oviduct.   When they are pushed ahead to form two lateral masses in the uterine cavity, the chromosomes are on the equatorial plate of the maturation division. After the second polar body is expelled in the central pouch of the uterus, embryonic development takes place in the uterine cavity as far as the gastrula stage (oviparous) or nauplius stage (viviparous) (Metalle and Ballardin, 1962).

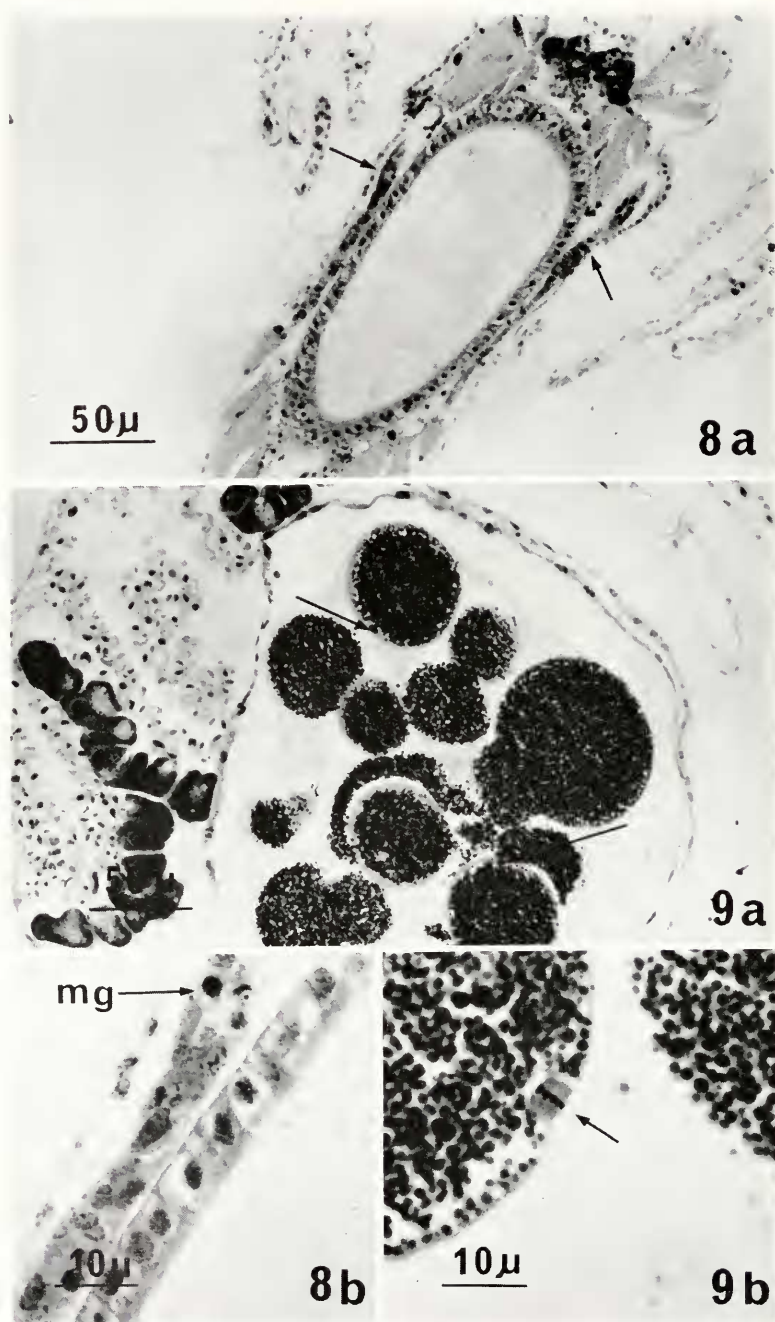The effect of γ-rays on the ovary of adult shrimps largely depends on the

FIGURE 8.   Regenerating ovary of a young *Artemia* 2 weeks after irradiation with 2 krads. Mitotic oogonia (mg) and newly proliferated oogonia are found; a: × 300, b: × 1200.

FIGURE 9.   Oocytes in the uterus of an adult *Artemia* 2 weeks after irradiation with 2 krads.   Arrows show normal metaphase of maturation division.   The cells were in an early meiotic stage at the time of irradiation; a: × 300, b: × 1200.

stage of development of the oocytes at the time of irradiation. In *Artemia* receiving 2 krads of γ-rays, oocytes in various stages (pre-meiotic, meiotic prophase and the first maturation division) and eggs undergoing early cleavage in the uterus remained apparently intact during the first day following irradiation. Two weeks after irradiation, most growing oocytes present at the time of irradiation had reached the mature stage, and they subsequently underwent normal maturation division (Figs. 9a, 9b).

In contrast to this, oocytes in adults receiving 5 krads were damaged to various degrees depending on their stage of development (Figs. 10, 11). Generally, oocytes at the pre-meiotic stage were severely damaged, and their nuclei became
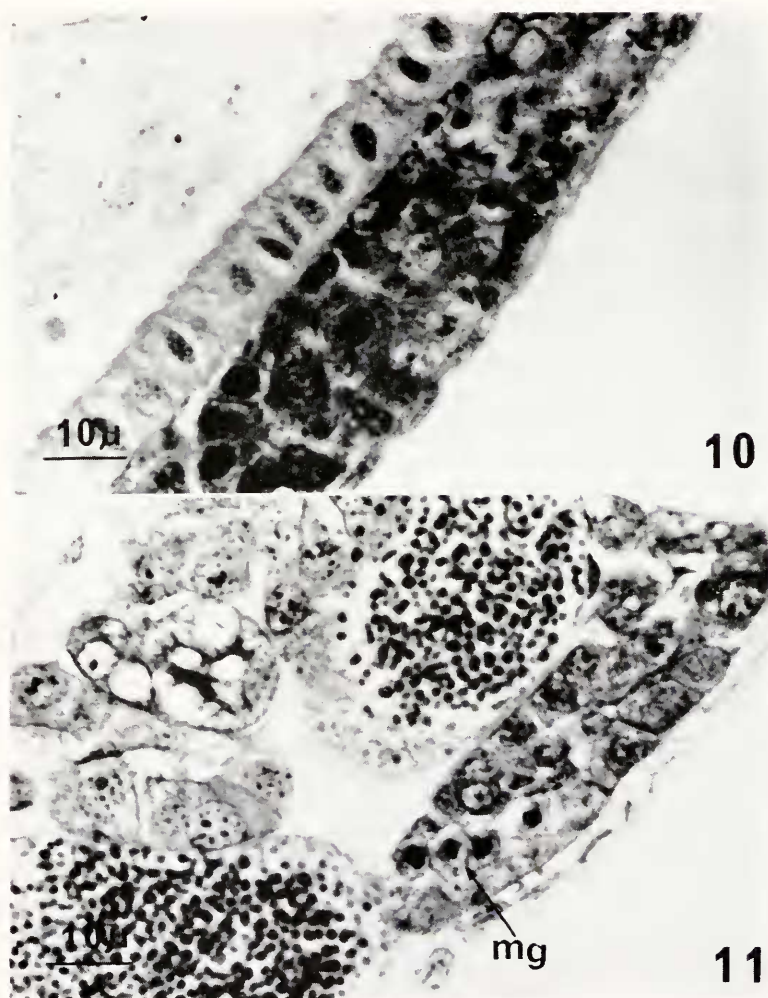

FIGURE 10. A portion of an ovary of an adult *Artemia* 1 day after irradiation with 5 krads. Pre-meiotic oocytes are pycnotic, × 1200.

FIGURE 11. A portion of an ovary of an adult *Artemia* 1 day after irradiation with 5 krads. Four metaphasic oogonia (mg) are shown in the neighborhood of growing oocytes. Figures 10 and 11 are different areas of an ovary in the same individual, × 1200.

pycnotic within one day after irradiation (Fig. 10). On the other hand, growing oocytes did not show any immediate effects of radiation (Fig. 11), and the maturation division and the nuclei of eggs undergoing cleavage were generally normal in appearance although some individuals having eggs with abnormal nuclei were occasionally found.

It should be pointed out that in adults as well as young shrimps, repopulation of the surviving oogonia was observed. That is, some dividing oogonia as well as oocytes were found at various times after irradiation in the ovary of adult shrimps receiving 2 or 5 krads (Figs. 11, 12a, 12b). In the ovaries of unirradiated adult shrimps, this situation is rarely observed. However, whether or not these
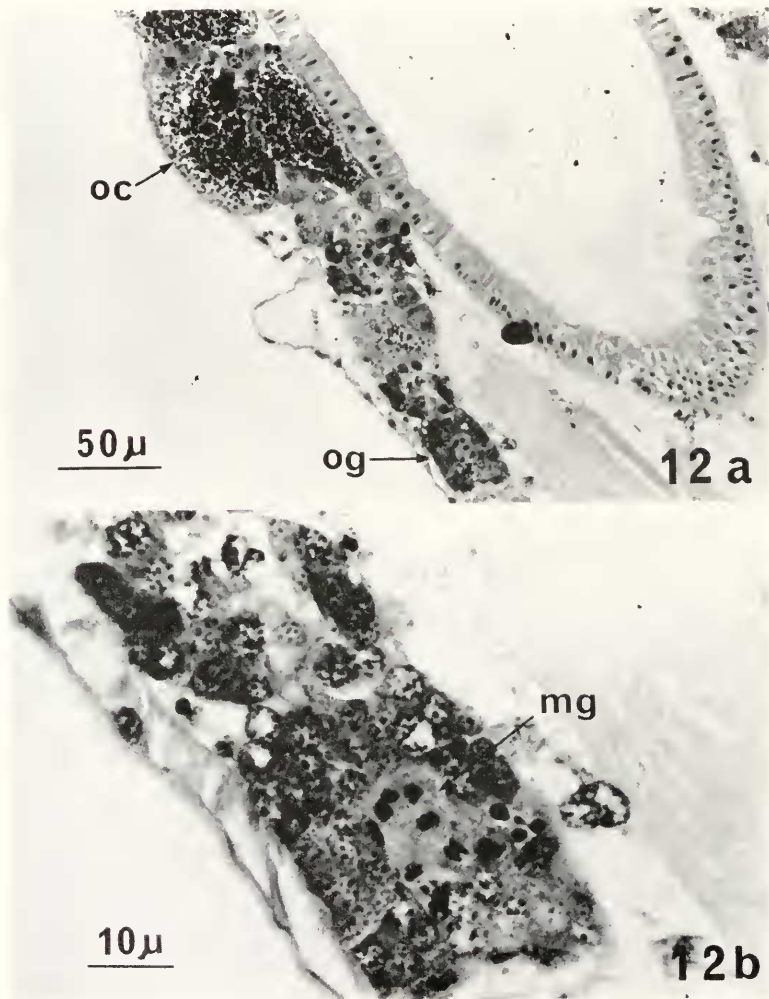

FIGURE 12. Ovary of an adult *Artemia* 2 weeks after irradiation with 2 krads showing some mitotic oogonia (mg); a: × 300, b: × 1200.

newly formed oogonia become functional oocytes has not been determined in these experiments.

<div align="center">Discussion</div>

In a previous paper, the author reported that if radiosensitivity was determined on the basis of hatchability, it was very low, the 50% hatching dose being about 400 krads (Iwasaki, 1964, 1965), and that although 200 krads of γ-rays had no significant effect upon the hatchability of the eggs, it greatly reduced the viability of nauplii derived from them (Iwasaki, Maruyama, Kumamoto and Kato, 1971). The author also reported that in squashed preparations of 1-day-old nauplii derived from eggs receiving 200 krads or less of γ-rays the number of mitotic cells did not decrease below control levels, but that pycnotic cells were found at doses as low as 20 krads (Iwasaki *et al.*, 1971). These cytological data obtained by squashed preparations are in agreement with the histological observations in the present experiments, that is, many normal mitoses were found in nauplii hatching from eggs irradiated with 150 krads although pycnotic cells were observed in nauplii derived from eggs irradiated with as low a dose as 25 krads.

According to Bowen (1963), a dose of 50 kR administered to encysted dry eggs did not kill during embryonic development but instead killed during later developmental stages (through the eighth instar). In our experiment, some nauplii derived from the dry eggs irradiated with 50 to 100 krads did reach maturity, but their ovaries contained pycnotic oocytes in addition to oocytes with normal nuclei It may be expected on the basis of these histological observations that these doses would result in reduced fecundity of *Artemia,* although studies of fecundity and fertility were not performed in the present experiment.

Compared to dry eggs, young shrimps are very sensitive to radiation; even a dose of 2 krads results in severe damage to the ovary and the eventual life-shortening of the shrimp. This radiosensitivity may be correlated with high mitotic activity of the oogonia. Because, it is in this stage that oogonia undergo repeated mitotic divisions and rapidly increase in number. The results are similar to those found in the rat by Beaumont and Mandl (1962) and Beaumont (1965), who concluded that oogonia are intrinsically more radiosensitive than oocytes based on a comparison of the numbers of germ cells recorded 24 hours after irradiation.

Studies with a number of organisms using a variety of endpoints have shown that the radiation sensitivity of the germ cells depends upon their stage in meiosis at the time of exposure. In rats, the last pre-meiotic division is highly sensitive to radiation whereas meiotic prophase is relatively less sensitive (Beaumont and Mandle, 1962). In a recent review, Baker (1971) states that in mammals, sensitivity increases during the final pre-meiotic division, and the oocyte subsequently becomes increasingly resistant to radiation as it progresses from leptotene to pachytene. In *Habrobracon,* metaphase of meiosis is 20 times more sensitive to x-rays than prophase I for the induction of dominant and recessive lethal mutations (Whiting, 1945). Similarly, in a parthenogenetic stick insect, oocytes in the first metaphase are about 8 times more sensitive at the LD_{50} level than those in the first prophase (Pijnacker, 1971). In the silkworm, germ cells in the first meiotic division are more resistant to lethal damage than those in the second meiotic division (Murakami, 1971). Metalli and Ballardin (1962, 1972) and Cervini and Giavelli

(1965) have investigated the relationship between meiotic stage sensitivity and dominant lethality as the basis of hatching inhibition in *Artemia;* hatchability was lowest in the earliest prophase stage scored and increased througout prophase reaching a maximum at metaphase. That is, oocytes were more sensitive in prophase than in metaphase. Based on histological evidence, the present experiments indicate that pre-meiotic oocytes are much more sensitive to γ-rays than growing oocytes or oocytes at maturation division (metaphase I). It may be significant that the former stage is characterized by a high rate of DNA synthesis but not the latter one (Iwasaki, 1970). Since no correlation was observed between genetic damage and cell killing, our results are not directly comparable to those of Metalli and Cervini. However, the pattern of radiosensitivity for cell killing during meiosis of *Artemia* oocytes was the same as that observed for the production of dominant lethals.

Squire (1970) has proposed that the *Artemia* ovary normally contains a permanent oogonial stem-cell component which contributes to each successive brood. In the ovary of adult *Artemia* irradiated with 2 or 5 krads, we observed some oogonia entering mitosis after irradiation, resulting in an increase in the number of germ cells. Therefore, some oogonia having the potential to undergo mitosis persist in the ovary of adult shrimp, lending support to Squire's proposal.

Grosch and Erdman (1955) reported that *Artemia* females die within several days after irradiation with 150 kR of x-rays and that the sterility dose for the female is 2 to 3 kR. Recently, Squire (1970) has also reported on female reproductive performance. According to him, sterility was almost complete after 5 kR of γ-rays, and the number of broods was significantly decreased at doses of 2 kR and above. Judging from our histological observations, it may be suggested that at doses of 5 krads or less the first brood derived from oocytes which were in post-meiotic or early cleavage stages at the time of irradiation may be laid normally. The subsequent brood, however, may not be produced or may be reduced in number, even though partial regeneration of oogonia has occurred.

Summary

Histological changes in the gonads of γ-irradiated *Artemia* were studied. Three different developmental stages were irradiated; dry eggs, immature young and adults. Normal growing oocytes and some pycnotic oocytes were found in the ovaries of quite a few surviving adult animals derived from dry eggs irradiated with 100 krads of γ-rays. Oogonia and primary oocytes in ovaries of immature *Artemia* irradiated with 5 krads of γ-rays became pycnotic within one day after irradiation, and became greatly reduced in number in 4 days. However, 10 to 14 days after irradiation, some surviving oogonia began to undergo mitosis. In adult animals, although pre-meiotic oocytes were damaged growing oocytes did not show any immediate damage after dose of 5 krads of γ-rays. Seven days after irradiation, they reached the mature stage, and normal maturation division occurred.

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